REMARKS

With entry of the instant amendment, claims 20, 27 - 29 and 41 - 46 are pending in the application. Claims 20, 27 and 41 have been amended. Claims 21 - 26 have been canceled. Additionally, claims 1 - 19 and 30 - 40 were previously canceled. New claims have not been added and new matter has not been introduced by said amended claims.

Claim Amendments.

Claim 20 has been amended to incorporated numerous elements of now canceled claims 21 - 26. Further the phrase, "capable of utilizing 2-keto-L-gulonic acid (KLG) as a carbon source to produce ascorbic acid or an ascorbic acid stereoisomer" has been moved from the preamble of the claim. The dependency of claim 27 has been changed to claim 20.

Rejections under 35 U.S.C. §112, first paragraph.

Claims 20 - 25 have been rejected as non-enabled by the specification.

In the examples, Applicant has provided two yeast species (*Candida blankii* and *Cryptococcus dimennae*) which are not only capable of growing solely on 2-keto-L-gulonic acid (KLG) as a carbon source, but also capable of using KLG to produce ascorbic acid (ASA). To expedite prosecution of the application, Applicant has amended the claims to define the yeast as members of the family Cryptococcaceae. Moreover, following the teachings presented in the application, one of ordinary skill in the art would be able to determine if a recombinant yeast of the Cryptococcaceae family was capable of growing on KLG as a sole carbon source, and further whether said yeast was capable of using KLG to produce ASA. As observed from the example, *Candida shahatae* was capable of using KLG as a sole carbon source but was not able to produce ASA from KLG.

Applicant submits the amendment to said claims should obviate the rejections presented under section 112, first paragraph.

Rejections under 35 U.S.C. §103(a).

Claims 20 - 29 and 41 - 43 have been rejected as unpatentable over Murakawa et al. (Agric. Biol. Chem. Vol 41(9):1799 - 1800); Hardy et al. (USP 4,945,052) and Anderson et al., (USP 5,032,514). The Applicant respectfully traverses said rejection.

The Examiner states at pages 7 and 8 of the Office Action,

> "Murakawa et al. teach the production of ascorbic acid from non-recombinant yeasts using a wide variety of sugars including glucose. However, the yields appear to be low. Thus, it appears that it was known in the art that 2,3-DKG occurs among yeasts and that they are capable of producing ASA.
>
> Anderson et al. teach a metabolic pathway for engineering an increased production of ascorbic acid intermediates by using recombinant technology by transfer of genes responsible for the bioconversion of a six carbon sugar such as glucose to 2-KLG which is next oxidized to ascorbic acid using the very same enzymes taught in this instant application. ..... However, the reference does not teach the utilization of yeasts for the fermentative method or the bioconversion method. The reference does teach that the recombinant technique can be used using any appropriate host cells.....
>
> Hardy et al. teach the production of vitamin C precursors, 2,5-DKG, in genetically modified microorganisms including several bacteria, fungi and yeast (col.5, last para) by transforming yeast host cells using a vector expressing the enzyme required for converting 2,5DKG to 2-KLG. The reference teaches recombinant methods and suggests the use of a list of microorganisms and mammalian cells as host cell."

Murakawa et al. suggest a hypothetical pathway for the biosynthesis of D-erythroascorbic acid (D-EasA) in the yeast *Candida utilis* starting from D-arabinose. However, more specifically, with regard to the Examiner's comment about Murakawa et al., Applicant has stated in the present specification at page 1, lines 29 - 35, "The presence of ASA in yeasts has been reported...." However, as stated in the Summary of the Invention, "The present invention is based in part upon the unexpected discovery that multiple members of yeast which are able to grow on ascorbic acid or iso-ascorbic acid as a sole carbon source are capable of utilizing KLG as a sole carbon source to produce ascorbic acid." Murakawa et al. does not disclose or suggest that a recombinant member of the Cryptococcaceae family would be able to utilize KLG as a sole carbon source to produce ASA.

Andersen et al. is directed to an improved process for converting glucose to KLG. The reference teaches a process of converting a common metabolite such as glucose to KLG in a single recombinant bacterial host cell by introducing a polynucleotide into the bacterial host which encodes 2,5-DKG reductase. The reference neither teaches or suggests the introduction of a heterologous nucleic acid encoding 2, 5-DKG reductase in yeast cells nor the

utilization of yeast for a method of producing ASA.

The Examiner has taken the position that Hardy et al. is the critical reference (see page 9 of the Office Action). Hardy et al. is also concerned with the conversion of a carbon source such as glucose to 2-KLG in a single fermentation step in a bacterial microorganism. More specifically, Erwinia is the host for a heterologous polynucleotide encoding a 2,5-DKG reductase. The Examiner emphasizes that the reference teaches transforming yeast host cells using a vector expressing the enzyme required for converting 2,5 DKG to KLG (column 5, last para). However, this is the only mention of yeast cells in the reference and there is no specific mention of yeast hosts or strains. Moreover, Applicant asserts this is not the claimed invention.

Applicant contends that the disclosure of Hardy et al. is just as deficient as the Anderson et al. disclosure. Even if Hardy et al. is combined with Anderson, Applicant fails to envision what invention might be made obvious, but it is not the claimed invention.

At best, the combination of references may suggest to one of skill in the art that a yeast host cell could be used for expression of a heterologous 2,5-DKG reductase. However, even if this were the case, the expression of a heterologous 2,5-DKG reductase in a yeast cell is not the invention. The invention concerns the capability of a member of the Cryptococcoccus family to grow on KLG as a single carbon source and the ability of the same host to produce ASA.

These cited references do not suggest a recombinant yeast which is capable of growing on KLG as a sole carbon source to produce ASA and producing ASA or an ASA stereoisomer wherein the yeast comprises a heterologous nucleic acid encoding a glucose dehydrogenase, a gluconate dehydrogenase or a KDG dehydrogenase and/or a heterologous nucleic acid encoding a 2,5DKG reductase.

For a rejection under 35 U.S.C. §103 (a) to be proper, the Examiner must show a) that each element of a claim is disclosed or suggested in the prior art, b) that the prior art provides the motivation to combine and modify the disclosures of the cited references to obtain the claimed invention and c) that the skilled artisan would have a reasonable expectation of success in obtaining the invention. Applicant asserts that these criteria have not been met because there is no disclosure or suggestion found in the cited references either alone or in combination of a recombinant yeast capable of using KLG as a sole carbon source for the production of ASA or an ASA stereoisomer wherein the yeast comprises a heterologous polynucleotide as claimed by

Applicant. Moreover, there is no motivation provided to modify the cited references to obtain the claimed invention.

Additionally the Examiner has rejected Claims 44 - 46 as unpatentable over Murakawa et al. (Agric. Biol. Chem. Vol 41(9):1799 - 1800); Hardy et al. (USP 4,945,052); and Anderson et al., (USP 5,032,514) as applied to claims 20 - 29 above and further in view of Saito et al. (Appl. Environ. Microbiol. 1997 vol. 63(2)454-460). Applicant respectfully traverses said rejection. The references of Murakawa et al., Andersen at al., and Hardy et al., have been discussed above. While the Saito et al. reference may disclose the cloning for L-sorbose dehydrogenase and recombinant bacteria cells capable of using sorbitol for production of KLG, this is not the claimed invention. The claimed invention is not simply a recombinant yeast comprising a heterologous gene encoding a L-sorbose dehydrogenase useful for converting sorbitol to KLG.

Applicant respectfully requests the withdrawal of all pending rejections and asserts the instant pending claims are in condition for allowance. Allowance of claims 20, 27 - 29 and 41 - 46 is kindly requested.

Respectfully submitted,

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Enc: Clean copy of pending claims

CLEAN COPY OF PENDING CLAIMS:

20. (Currently amended): A recombinant yeast comprising either one or both of

a) a heterologous nucleic acid encoding a glucose dehydrogenase, a gluconic acid dehydrogenase or a 2-keto-D-gluconic acid dehydrogenase,

and

b) a heterologous nucleic acid encoding a. 2,5 -diketo-L-gluconic acid (2,5-DKG) reductase,

wherein the yeast is a member of the family *Cryptococcaceae* and is capable of utilizing 2-keto-L-gulonic acid (KLG) as a sole carbon source to produce ascorbic acid or an ascorbic acid stereoisomer.

27. (Currently amended): The yeast of Claim 20 wherein the yeast is a *Candida* or *Cryptococcus*.

28. (Previously amended): The yeast of Claim 27 wherein the yeast is *Candida blankii*.

29. (Previously amended): The yeast of Claim 27 wherein the yeast is *Cryptococcus dimennae*.

41. (Currently amended): A recombinant yeast comprising either one or both of

a) a heterologous nucleic acid encoding a glucose dehydrogenase and

b) a heterologous nucleic acid encoding a 2,5 -diketo-L-gluconic acid (2,5-DKG) reductase

wherein said yeast is *Candida blankii* or *Cryptococcus dimennae* capable of utilizing 2-keto-L-gulonic acid (KLG) as a carbon source to produce ascorbic acid or an ascorbic acid stereoisomer and capable of converting glucose to KLG and utilizing the KLG to produce ascorbic acid or an ascorbic acid stereoisomer.

42. (Previously added): The recombinant yeast of Claim 41 wherein said yeast is *Candida blankii*.

43. (Previously added): The recombinant yeast of Claim 41 wherein said yeast is *Cryptococcus dimennae*.

44. (Previously added): A recombinant yeast capable of utilizing 2-keto-L-gulonic acid (KLG) as a carbon source to produce ascorbic acid or an ascorbic acid stereoisomer, said yeast comprising at least one heterologous nucleic acid encoding a L-sorbose dehydrogenase, a D-sorbitol dehydrogenase, a L-sorbosone dehydrogenase or a galactose dehydrogenase, wherein said yeast is *Candida blankii* or *Cryptococcus dimennae* and is capable of converting sorbitol to KLG and then utilizing the KLG to produce ascorbic acid or an ascorbic acid stereoisomer.

45. (Previously added): The recombinant yeast of Claim 44 wherein said yeast is *Candida blankii.*

46. (Previously added): The recombinant yeast of Claim 44 wherein said yeast is *Cryptococcus dimennae.*